1. (25 points) An antibody has been discovered that has a metal dependence for $Fe^{2+}$ binding. Upon further investigation, it is determined that the antibody has three [redacted] $Fe^{2+}$ binding sites per antibody molecule. An equilibrium dialysis experiment is done to determine the equilibrium binding constant, K. The following data are measured after equilibrium at pH 7 in a 1 M KCl solution:

Total concentration of protein inside the dialysis chamber = 10.0 mM
Concentration of $Fe^{2+}$ outside the dialysis chamber = 1.0 μM
Ratio of total $Fe^{2+}$ inside to $Fe^{2+}$ outside = 957

units = +1
[bound] = +3
$\nu$ = +3
Scatchard Eqn +1
[A free, in] = +3
K = +3

(a) Calculate the equilibrium constant, K, per binding site.

$[Fe^{2+}{}_{\text{free, inside}}] = 1\ \mu M$

$\nu = \frac{[\text{bound}]}{[AB]} = \frac{956\ \mu M}{10 \times 10^3\ \mu M} = 0.0956$

$\frac{\nu}{[A]} = K[N - \nu]$

$K = \frac{\nu}{[A]}\frac{1}{N-\nu} = \frac{0.0956}{[1\ \mu M][3-0.0956]} = 0.0329\ \mu M^{-1} = 32{,}915\ M^{-1}$

$3.3 \times 10^4\ M^{-1}$

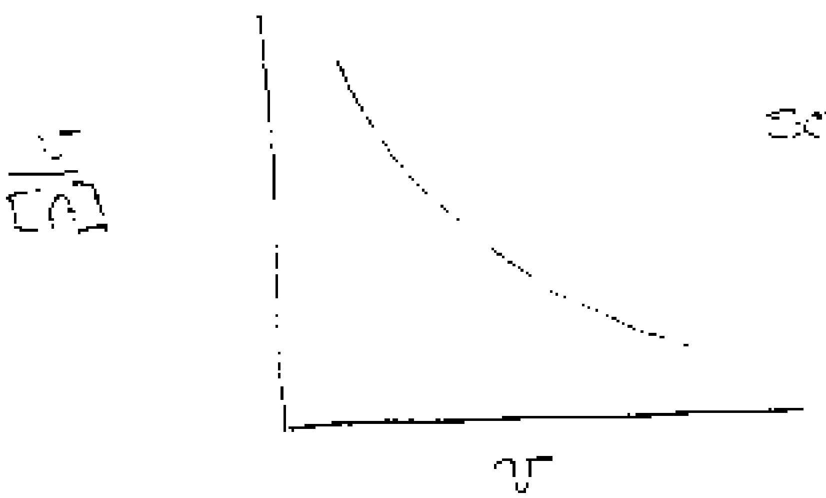
(b) The experiment was repeated with increasing concentrations of $Fe^{2+}$. It was found that the value of K varied with $Fe^{2+}$ concentration. What are the possible reasons for this?

sites are not independent (+3)

&/or

sites are not identical (+3)

(c) Draw a cartoon picture of what the Scatchard plot would look like. Do not include values - label the axes and show a general trend consistent with the above information and what information the plot provides.




label axes +2
some sort of curve of negative slope +3

2. (25 points) A similar antibody is observed to be able collect light. These particular antibodies aggregate into very large complexes. To analyze the nature of these proteins some electrophoresis and osmotic pressure measurements were made. On a polyacrylamide gel run with SDS and under denaturing conditions one band was observed at approximately 26 kiloDaltons. $[\text{kD} = 1000\ \text{g} \cdot \text{mol}^{-1}]$

Osmotic pressure measurements were made at 37°C using a membrane which would let molecules less than 2 kD pass through, but not larger. When a solution of 10mg of protein per 1 ml of dilute buffer was measured, the osmotic pressure was found to be $5.81 \times 10^{-4}$ atm. However, when the chaotropic salt sodium thiocyanate was added to a concentration of 1 M the osmotic pressure increased to $4.68 \times 10^{-3}$ atm.

Find the effective molecular weight with and without sodium isothiocyanate. Find a consistent and complete explanation for both the electrophoresis data and the osmotic pressure.

$\Pi = \frac{W}{M}RT = cRT \; ; \quad c = [\text{mol} \cdot \text{L}^{-1}] \quad T = 310\ \text{K} \quad R = 0.08205\ \frac{\text{L} \cdot \text{atm}}{\text{K} \cdot \text{mol}}$

$c = \frac{W}{M}$

(+3) $M = \frac{W}{\Pi}RT$

(+6) $M_{buffer} = \frac{(10\ \text{g} \cdot \text{L}^{-1})(0.08205\ \frac{\text{L} \cdot \text{atm}}{\text{K} \cdot \text{mol}})(310\ \text{K})}{5.81 \times 10^{-4}\ \text{atm}} = 437{,}788\ \text{g} \cdot \text{mol}^{-1} \cong 438\ \text{kD}$

(+6) $M_{NaSCN} = \frac{(10\ \text{g} \cdot \text{L}^{-1})(0.08205\ \frac{\text{L} \cdot \text{atm}}{\text{K} \cdot \text{mol}})(310\ \text{K})}{4.68 \times 10^{-3}\ \text{atm}} = 54{,}349\ \text{g} \cdot \text{mol}^{-1} \cong 54\ \text{kD}$

(+4) In NaSCN, the antibody is a dimer $\rightarrow \frac{54}{26} \cong 2$ (chaotropic salt tends to reduce aggregation)

(+4) In buffer, the dimers form an octamer $\rightarrow \frac{438}{54} \cong 8$

(+2) With SDS and under denaturing conditions, the antibody migrates as a monomer of 26 kD.

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$(\alpha-\alpha)_8 \rightarrow 8(\alpha-\alpha) \longrightarrow 16\alpha$ (where $\alpha \approx 26$ kD subunit)

Buffer

NaSCN breaks up noncovalent interactions

SDS/denaturing conditions may reduce disulfide bridge of dimer $\alpha-\alpha$

3. (25 points) For each of the following changes, state whether the sedimentation coefficient of the antibody will increase, decrease, remain the same, or it is impossible to tell. Give an equation and a one or two sentence explanation which supports your answer.

Points (a) The temperature of the aqueous suspension in increased from 20°C to 30°C.

$$S = \frac{m(1-\bar{v}_2\rho)}{f}$$

$$f = 6\pi\eta r$$

if $T\uparrow : \eta\downarrow : S\uparrow$

BUT . . .

$$f = \frac{kT}{D}$$

$T\uparrow : f\uparrow : S\downarrow$

rrect explanation no equations +5

uation with reasonable answer & conclusion +6

uation only +3

uation only & errors +1

rrect but no description of F: +9

change: +4

The discrepancy stems from the fact that there are other variables that also depend on temperature. (ex. D, ρ . . ) So it is impossible to tell

(b) The long axis of the protein (a rod) is decreased, keeping the protein volume and MW constant.

Points

$$f = 6\pi\eta r$$

$r\downarrow \quad f\downarrow \quad S\uparrow$

(c) $^{15}N$ is substituted for $^{14}N$ in the protein.

7 points

$m\uparrow \; . \; S\uparrow$ small effect

4. (25 points) The protein β-lactalbumin, 14 kD m.w., has been studied under different solution conditions by light scattering. At pH 7.0 and 40°C in a dilute solution the diffusion constant was found to be 11.50 x $10^{-7}$ $cm^2s^{-1}$. (Remember D=kT/f)

a) (8 points) If the viscosity of the solution is 0.0101 poise (1 poise = 1 g $cm^{-1}s^{-1}$), estimate the diameter of the protein assuming it is spherical in shape (in Å).

$f = 6\pi\eta r = \frac{kT}{D}$

(1) $r = \frac{kT}{6\pi\eta D}$

(4) $r = \frac{(1.38\times10^{-23} J K^{-1})(313 K)}{6\pi(.0101\ g\cdot cm^{-1}s^{-1})(11.5\times10^{-7}\frac{cm}{sec})}\left(\frac{1\ m}{100\ cm}\right)\left(\frac{1\ kg}{1000\ g}\right)$

$r = 1.97\times10^{-9} m = 19.7$ Å

(2) $d = 39.5$ Å

b) (8 points) At pH < 2 this protein becomes inactive and changes spectral characteristics. Under these conditions the diffusion constant was found to be 12.65 x $10^{-7}$ $cm^2s^{-1}$. How much change in volume occurred for the protein (in Å$^3$)? (assume all else remained the same).

$f = 6\pi\eta r$

$r \propto \frac{1}{D}$ (+2) $\underbrace{\frac{r_{new}}{r_{old}} = \frac{D_{old}}{D_{new}}}_{\text{or something similar}}$ $r_{old} = \left(\frac{11.5\times10^{-7}}{12.65\times10^{-7}}\right)(19.7\text{Å}) = 17.9$ Å (+2)

$V = \frac{4}{3}\pi r^3$ $\Delta V = \frac{4}{3}\pi(r_2^3 - r_1^3)$ (+2) $\Rightarrow \frac{4}{3}\pi((17.9)^3 - (19.7)^3)$

(+2) $\Delta V = -8000$ Å$^3$

$(8000\text{Å}^3)\left(\frac{1\ m^3}{10^{30}\text{Å}^3}\right) = -8\times10^{-21}$ mL

$\bar{V} = \frac{\frac{4}{3}\pi r^3}{m}$

c) (9 points) At intermediate pH there might be an equilibrium between these two forms of the protein. Calculate the ratio of sedimentation coefficients ($S_{high\ pH}/S_{low\ pH}$) for these two forms. State any assumptions you make.

$S = \frac{m(1-\bar{V}_2\rho)}{f}$ $\frac{S_{high}}{S_{low}} = \frac{(1-\bar{V}_2^h\rho)f_{low}}{(1-\bar{V}_2^l\rho)f_{high}}$ (+2) (+2) $\frac{f_{low}}{f_{high}} = \frac{\cancel{6\pi\eta}r_{low}}{\cancel{6\pi\eta}r_{high}} = \frac{r_{low}}{r_{high}} = .909$

$\bar{V}(high) = \frac{4}{3}\pi(19.7\text{ Å})^3\left(\frac{1\ cm^3}{10^{24}\text{Å}^3}\right) N_A\left(\frac{\text{mol}}{14000\ g}\right) = 1.38\ \frac{cm^3}{g}$

$\bar{V}_2(low) = \left(\frac{r_{low}}{r_{high}}\right)^3 \bar{V}_2\ high = 1.04\ \frac{cm^3}{g}$

$\bar{V} = \frac{4}{3}\pi r^3$ ratio $= \left(\frac{1-(1.38)(1.04)}{1-(1.04)(1.04)}\right)(.909) = 4.94$ (+3)